STIMULATION OF THE RELEASE OF PROSTAGLANDINS FROM POLYMORPHONUCLEAR LEUKOCYTES BY THE CALCIUM IONOPHORE A23187

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Received 21 November 1977
Revised version received 16 December 1977

1. Introduction

The coincidental appearance of prostaglandins (PGs) and polymorphonuclear leukocytes (PMNL) at sites of acute inflammation has led to the suggestion that PGs found in inflammatory exudates are synthesized by PMNL [1–3]. In support of this hypothesis it has been shown that in vitro, phagocytizing PMNL release significant quantities of PGs, mainly PGE_1 [2,4]. The means by which PG release is stimulated by phagocytosis is not known but may involve the release of lysosomal phospholipases which in turn release free fatty acids including PG precursors [5]. There is some evidence that other factors such as activation of PG synthetase itself may also be involved [2].

Recently it has been shown that ionophores for divalent cations in the presence of Ca^{2+} can induce phagocytosis-associated responses in PMNL, including degranulation of lysosomes [6,7] and stimulation of oxidative metabolism [8.9]. In this communication we show that the ionophore A23187 in the presence of Ca^{2+} also induces PG release from PMNL and compare this release to the release of lysosomal enzymes.

2. Methods

Peritoneal exudate PMNL were obtained from male Wistar rats (150–200 g) which had been injected intraperitoneally with 6 ml sterile 2% sodium caseinate solution. Cells were collected 4–5 h after the injection, washed twice in calcium free Hank's balanced salt solution (HBSS), and resuspended in HBSS enriched with 0.2% glucose and containing the indicated Ca^{2+} concentrations. Microscopic examination of smears stained with Wright's stain showed that greater than 90% of the cells were PMNL, the remainder being macrophages and lymphocytes. Cell preparations contaminated with erythrocytes were not used. Platelets were not observed in these preparations by phase-contrast microscopy. Following various periods of incubation at 37°C with the indicated reagents, samples were immediately placed on ice, then centrifuged at 8000 × *g* for 4 min (4°C) and the cell-free supernatants removed.

PG levels in the cell-free supernatants were measured using a commercially available radioimmunoassay kit containing an anti-PGB_1 serum (Clinical Assays, Cambridge, MA). Inclusion of steps to remove protein from the supernates prior to assay were found not to alter the levels of PG measured and were not routinely included. An alkaline dehydration step was used to convert PGA_1 and PGE_1 in the samples to PGB_1 and the assay carried out as suggested by the manufacturer. Experimental data representing the total PGA_1 and PGE_1 levels are reported simply as ng 'PG' released/10^7 cells. That this immuno-reactive material actually represented newly synthesized PGs was verified by demonstrating that stimulation of release was completely inhibited by 28 μM indomethacin.

Enzyme release from PMNL is expressed as the percentage of the total enzyme activity released from cells incubated with 0.2% Triton X-100. β-Glucuronidase activity was determined as in [10]; lysozyme activity by as in [11]; and lactate dehydrogenase

(LDH) activity as in [12]. The activity of this latter enzyme was used as an indicator of cell damage.

Phorbol myristate acetate (PMA) was purchased from Midland Corp. (Brewster, NY). The ionophore A23187 was a generous gift from R. L. Hamill of Eli Lilly, (Indianapolis, IN). Stock solutions of both PMA and A23187 were prepared in dimethyl sulfoxide. Arachidonic acid and 8,11,14-dihomo-γ-linolenic acid were obtained from Nu Chek Prep. (Elysian, MN).

3. Results and discussion

The production of PGs by PMNL is markedly enhanced by the addition of fatty acids which are PG precursors but not by other fatty acids (table 1). These results are in accord with [13] where precursor fatty acid availability is indicated as the rate-limiting factor in PG production.

The ionophore A23187 is also capable of stimulating PG release above control levels, but its effect is not additive to the enhancement caused by the presence of dihomo-γ-linolenic acid (table 2). This suggests that the ionophore stimulates PG production, at least in part, by raising the levels of precursor fatty acids. This might occur as a result of ionophore-induced degranulation of lysosomes resulting in the release of phospholipases. We therefore investigated the relationship between lysosomal enzyme release and prostaglandin release. The close relationship between the stimulation by A23187 of lysosomal enzyme release and of PG release is shown by the similarity of the dependency of both processes on Ca^{2+} concentration (fig.1) and time (fig.2).

Table 1
Effect of fatty acids on PG release from resting PMNL

Fatty acid	PG release (ng/10^7 cells)
None (control)	0.4 ± 0.2
Dihomo-γ-linolenic acid	25.0 ± 2.1
Dihomo-γ-linolenic acid + indomethacin (28 μM)	2.8 ± 0.5
Arachidonic acid	22.2 ± 0.8[a]
Oleic acid	1.6 ± 0.4
Linoleic acid	0.8 ± 0.5
Stearic acid	0.7 ± 0.2

[a] This value is not corrected for the lower affinity that PGE_2, the prostaglandin product from this fatty acid, has for the antibody after its conversion to PGB_2 by alkaline dehydration

PMNL (4.2 × 10^6 cells/ml) were incubated at 37°C in the presence of 1 mM Ca^{2+} and the indicated fatty acids (10 μg/ml) for 30 min, after which PG levels in the cell-free supernatants were determined. Results represent mean ± standard error of mean (SEM) for triplicate determinations

Table 2
Effect of dihomo-γ-linolenic acid on A23187-stimulated PG release

Additions	PG release (ng/10^7 cells)
–	0.0 ± 0.1
A23187 (5 μM)	8.4 ± 1.1
Dihomo-γ-linolenic acid (10 μg/ml)	13.9 ± 0.8
A23187 (5 μM) + dihomo-γ-linolenic acid (10 μg/ml)	17.3 ± 1.2

PMNL (3.25 × 10^6 cells/ml) were incubated in HBSS containing 1 mM Ca^{2+} and the indicated reagents for 20 min after which PG levels in the cell free supernatants were determined

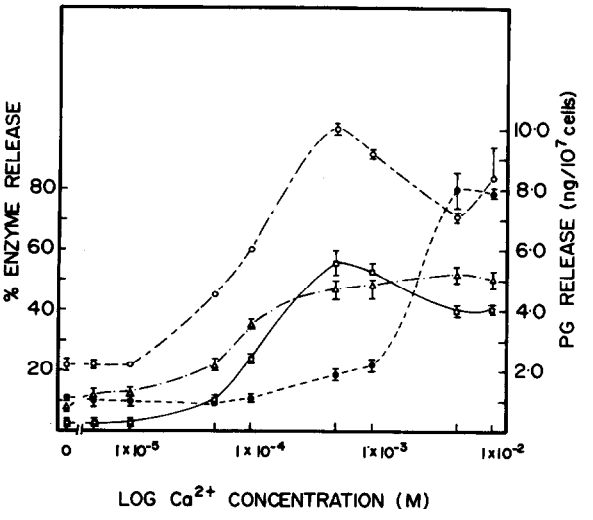

Fig.1. Dependence of A23187-induced PG and lysosomal enzyme release on Ca^{2+} concentration. PMNL (3.4 × 10^6 cells/ml) were exposed to 10 μM A23187 in the presence of the indicated concentrations of Ca^{2+} for 20 min. (○—○) PG release; (□—□) β-glucuronidase release; (△— ·△) lysozyme release; (●– –●) lactic dehydrogenase release. Results represent mean ± SEM for triplicate determinations.

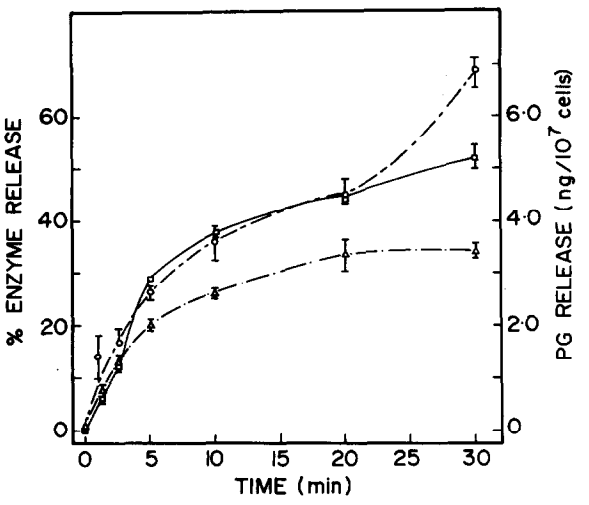

Fig.2. Time course of PG and lysosomal enzyme release from PMNL induced by A23187. PMNL (4.6×10^6 cells/ml) were incubated in HBSS containing 1 mM Ca^{2+} in the presence of 10 μM A23187. Samples were removed at the indicated times and the cell-free supernatants assayed for PG (○— -○), β-glucuronidase (□—□), and lysozyme (△— · △) content. Results represent the mean ± one-half range for duplicate determinations.

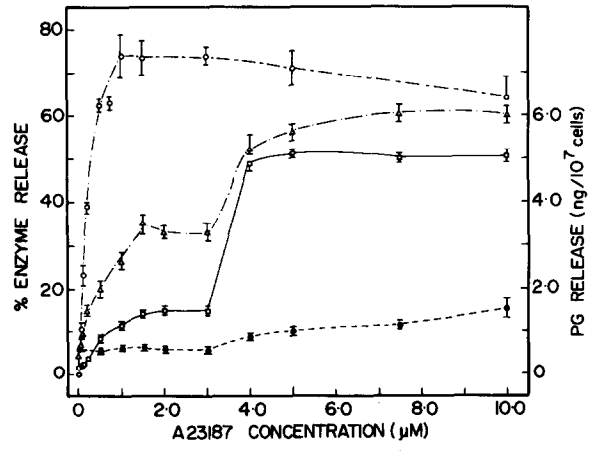

Fig.3. Dependence of PG and lysosomal enzyme release on A23187 concentration. PMNL (4.4×10^6 cells/ml) were incubated with the indicated concentrations of A23187 and 1 mM Ca^{2+} for 20 min after which cell-free supernatants were assayed for PG (○— -○), β-glucuronidase (□—□), lysozyme (△— · △) and lactic dehydrogenase (●- -●) levels. Results represent the mean ± SEM for triplicated determinations.

There are two types of lysosomal granules found in the PMNL – the primary or azurophil granules and the secondary or specific granules [14]. PMA has been shown to induce the release of enzymes from the specific granules only [15] and thus induce the release of lysozyme (found in both types of granules) but has little effect on β-glucuronidase (found only in azurophil granules). We have found that PMA does not induce PG release (data not shown). The relationship of PG release to lysosomal enzyme release can also be evaluated as a function of ionophore concentration. The results indicate that the release of PG occurs at ionophore concentrations below 1 μM and is largely independent of the major release of β-glucuronidase which occurs at ionophore concentrations of 3–5 μM. However, there is a small but statistically significant increase in β-glucuronidase release at ionophore concentrations of 0–3 μM. We must conclude therefore that PG release is independent of degranulation of specific granules (from the PMA results) and that it is either independent of, or very sensitive to, the degranulation of the azurophil granules.

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